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# Recent Mumps Outbreaks in Vaccinated Populations: No Evidence of Immune Escape

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**Recently, numerous large-scale mumps outbreaks have occurred in vaccinated populations. Clinical isolates sequenced from these outbreaks have invariably been of genotypes distinct from those of vaccine viruses, raising concern that certain mumps virus strains may escape vaccine-induced immunity. To investigate this concern, sera obtained from children 6 weeks after receipt of measles, mumps, and rubella (MMR) vaccine were tested for the ability to neutralize a carefully selected group of genetically diverse mumps virus strains. Although the geometric mean neutralizing antibody titer of the sera was lower against some virus strains than others, all viruses were readily neutralized, arguing against immune escape.**

Mumps is an acute, systemic, communicable viral infection characterized by swelling of one or both parotid glands, often accompanied by more serious complications, such as meningitis, pancreatitis, or orchitis. Mumps virus (MuV), a nonsegmented negative-strand RNA virus in the family *Paramyxoviridae*, encodes nine proteins from seven transcription units. The gene order is 3′-N-V/P/I-M-F-SH-HN-L-5′, representing nucleo- (N), V/phospho-/I (V/P/I), matrix (M), fusion (F), small hydrophobic (SH), hemagglutinin-neuraminidase (HN), and large (L) protein genes, respectively (28, 29). The functions of the viral proteins have been well described in the literature (9, 37). Briefly, the N, P, and L proteins are located within the virion and are responsible for genome transcription and replication. The M protein, also located internally, is involved in virion assembly and budding and may also regulate genome transcription and replication. The F and HN glycoproteins, present on the outer surface of the viral envelope, are responsible for virus-to-cell attachment and virus-to-cell and cell-to-cell fusion. The SH and V proteins are nonstructural accessory proteins involved in evasion of the host antiviral response. The role of the I protein in the life cycle of the virus is not known.

Prior to implementation of mumps immunization programs, more than 90% of most populations had serologic evidence of exposure to MuV by 15 years of age (11, 44). Within a decade of the 1967 implementation of mumps vaccination in the United States, disease incidence declined from greater than 100 cases reported per 100,000 population to less than 10 cases per 100,000 (12). By 2001, the disease was nearly eliminated, with less than 0.1 case per 100,000 (43). Similar success in the control of mumps has been achieved in other countries (32, 50, 58); however, over the past 6 years, mumps has made a resurgence globally, including in the United States, which recently experienced its largest outbreak since 1987 (5, 7, 13, 14, 19, 42, 49, 52, 53, 62). Whereas mumps was historically a disease of childhood, these outbreaks predominantly involved young adults, nearly all of whom had a history of vaccination during childhood, most with the recommended two-dose schedule. While these data are suggestive of waning immunity, it has also been postulated that antigenic differences between the vaccine and outbreak strains may allow for vaccine escape (20, 45). Indeed, viruses isolated from recent outbreaks cluster into genotype groupings distinct from those of the vaccine strains used. With few exceptions, genotype G strains have been isolated from cases in the Western hemisphere (27), genotype J and F from the Asia-Pacific region (5, 16), and genotype H from the Middle East (3, 33), whereas the mumps vaccines used in these countries contain predominantly genotype A Jeryl Lynn (JL)-based vaccines and to a lesser extent the genotype B Urabe-AM9 vaccine and the yet to be assigned genotype Leningrad-Zagreb vaccine.

To comprehensively investigate the possibility that certain mumps virus strains may be insensitive to vaccine-induced antibody, we sought first to identify viral protein targets of neutralizing antibody and then to construct phylogenetic trees based on the amino acid sequences of these proteins. A representative virus member from each grouping would then be used in plaque reduction neutralization (PRN) assays with sera (kindly provided by Merck and Co.) obtained from 96 4- to 6-year-old children 6 weeks after receipt of a second dose of the measles, mumps, and rubella (MMR) vaccine containing the JL mumps virus strain (51).

Although it is clear that the MuV HN protein is a target of neutralizing antibody (21, 35, 40, 48, 59), the virus-neutralizing capacity of antibodies directed against other MuV proteins has not been adequately investigated. Here, reverse genetics techniques were used to construct full-length cDNA plasmids encoding different combinations of viral N, V/P/I, L, F, and HN proteins derived from two genetically disparate MuV strains, the genotype A JL vaccine virus and the genotype H 88-1961 (here referred to as 88) wild-type virus (4). Antibodies directed against the nonessential SH protein have not been detected in human sera; thus, it is unlikely that such antibodies, if they exist, play an important role in antibody-mediated virus neutralization. The SH protein was therefore excluded from this analysis. The role of the M protein was not evaluated. The genetic makeup of the eight recombinant viruses used for the analysis is shown in Fig. 1.

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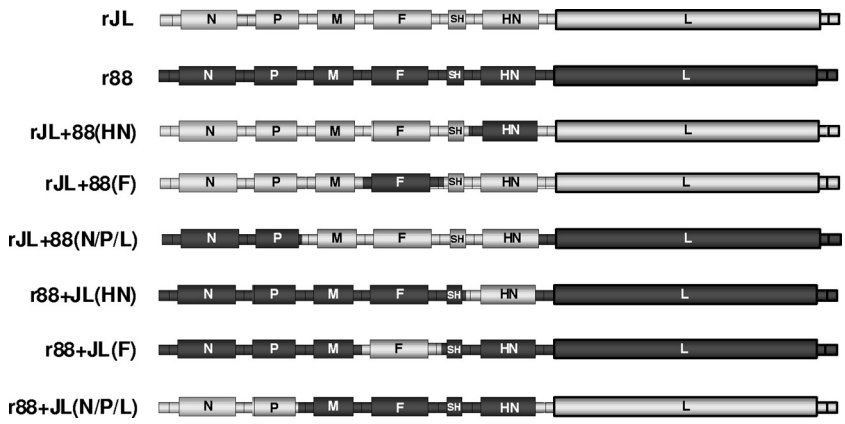


**FIG 1** Genome structure of recombinant viruses. Boxed elements shown in gray or black denote Jeryl Lynn (JL)- or 88-1961 (88)-derived sequences, respectively. Smaller boxes between open reading frames delineate untranslated regions. As per convention, the V/P/I gene is referred to here as the P gene. The construction of these viruses is described elsewhere (56).

A subset of the 96 serum specimens ($n = 10$, preselected for titer range) were tested for their relative neutralizing capacity against these eight recombinant viruses in PRN assays performed as described earlier (54). Results are shown in Fig. 2. All comparisons were performed using log-transformed data and the Student's $t$ test ($\alpha = 0.05$). As expected, replacement of the JL HN gene with that of 88 [rJL+88(HN)] or vice versa [r88+JL(HN)] yielded geometric mean titers (GMTs) that were significantly different than those of the parental viruses (all $P$ values were $<0.001$), confirming the HN protein as a major target of neutralizing antibody. In contrast, replacement of the JL F gene with that of 88 [rJL+88(F)], or vice versa [r88+JL(F)] yielded GMTs not statistically different from those measured against the parental viruses ($P$ value of 0.06 or 0.385, respectively), suggesting that the MuV F gene does not play a significant role in the neutralizing antibody response. This is consistent with findings by others who were unable to achieve virus neutralization with anti-MuV F protein antibodies (47, 60, 63), although one group reported that serum from hamsters infected with vaccinia virus expressing the MuV F protein was capable of virus neutralization *in vitro* (34). No effect on neutralization was seen with replacement of the N, V/P/I, and L genes [rJL+88(N/P/L) and r88+JL(N/P/L); $P$ values of 0.556 and 0.663, respectively], a finding that was perhaps not surprising considering the likely inaccessibility of these internally expressed proteins to antibody. Nonetheless, neutralization by antibodies specific for internally expressed proteins has been reported for other viruses (22, 39, 41). Although Western blot analysis revealed differences in viral protein content between the different viruses, levels of protein expression did not correlate with susceptibility to neutralization (data not shown).

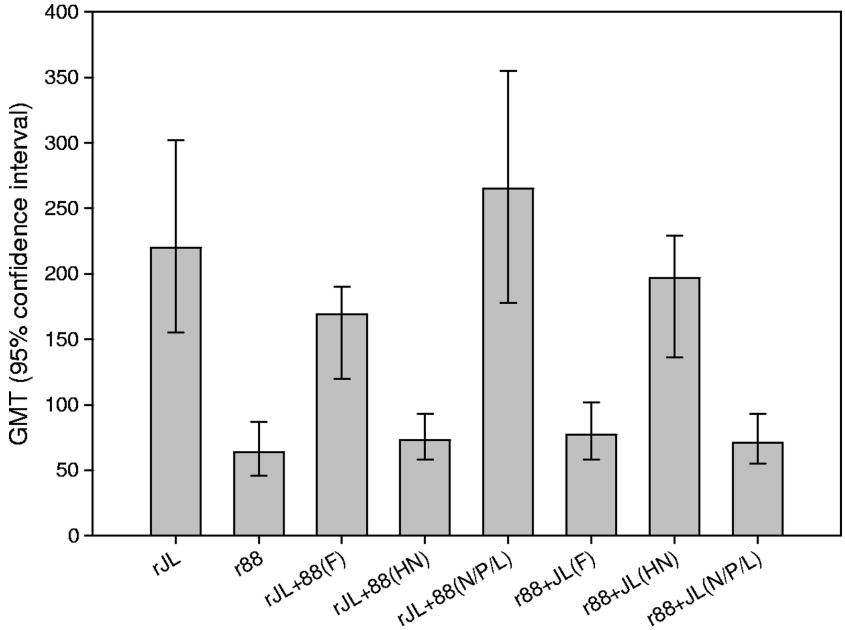


**FIG 2** Plaque reduction neutralizing antibody titer (GMT) calculated for 10 sera against eight different virus constructs. Bars indicate upper and lower bounds of the 95% confidence intervals. PRN titers are expressed as the reciprocal of the highest serum dilution factor required to neutralize at least 50% of the challenge virus PFU.

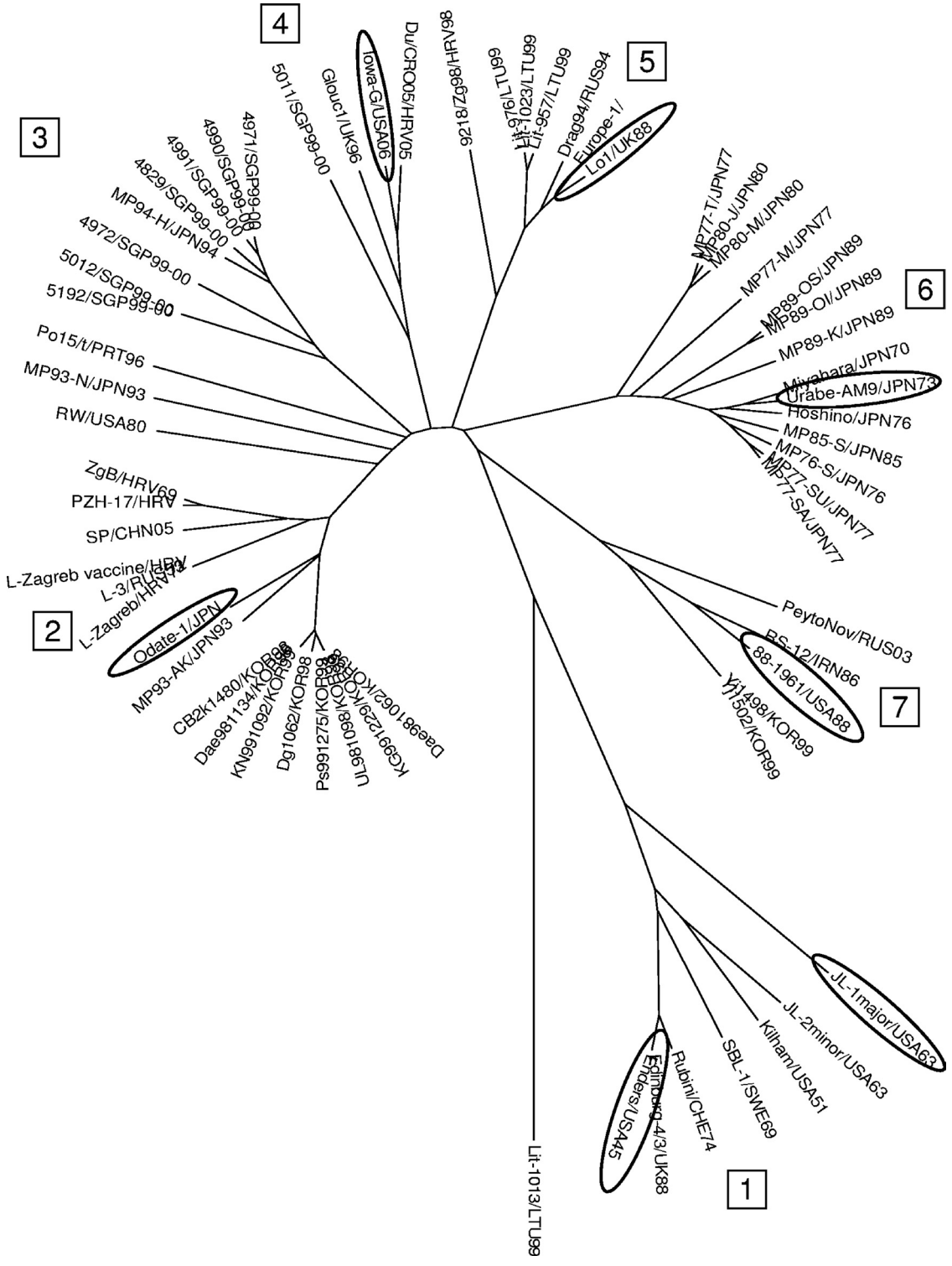


**FIG 3** Phylogenetic tree constructed using full-length HN amino acid sequences for 65 unique MuV strains obtained from NCBI Entrez databases. Virus strains selected for the study are indicated. These are vaccine strains Jeryl Lynn/USA63 (the major MuV component in M-M-R II [2]) and Urabe-AM9/JPN73 (64) and clinical isolates Enders/USA45 (30), Odate-1/JPN (55), Iowa-G/USA06 (54), Lo1/UK88 (1), and 88-1961/USA88 (4). The arbitrary group numbers are boxed.

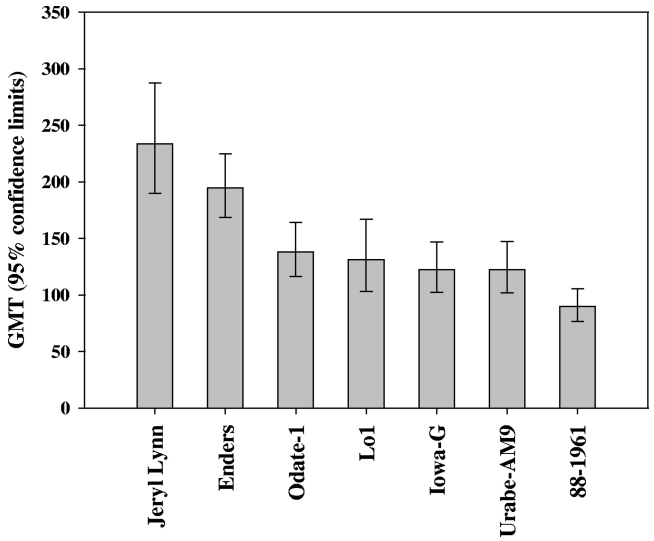


**FIG 4** GMTs of sera from MMR vaccines tested against seven different MuV strains. Bars indicate upper and lower bounds of the 95% confidence intervals.

Based on the demonstration of the HN protein as the major player in virus susceptibility to antibody-mediated neutralization, all unique mumps virus strains for which the full-length HN amino acid sequence was available in the NCBI databases (http://www.ncbi.nlm.nih.gov) were used to construct a phylogenetic tree using the freeware program MEGA v3.1 (36) using the unweighted pair-group method with arithmetic means (UPGMA) (26). The resulting tree showed seven distinct clusters, arbitrarily labeled as groups 1 to 7 (Fig. 3). Similar clustering of viruses was achieved when the analysis was repeated using the SH gene nucleotide sequence (data not shown). One virus was selected from each HN grouping, with the exception of group 1, for which two viruses were chosen to allow assaying of both the homologous vaccine strain (JL) and a different group 1 virus. No viruses representing group 3 were available. Thus, a total of seven MuVs were tested.

The GMTs of the 96 serum samples tested against the 7 MuV strains are presented in Fig. 4. All sera neutralized all viruses. Not surprisingly, the highest titers were measured against JL (the immunizing agent). No statistically significant differences were seen between the anti-JL and anti-Enders/USA45 GMTs (233 versus 195, $P = 0.166$, Mann-Whitney rank sum test), consistent with the two viruses belonging to the same HN phylogenetic group. In contrast, the anti-JL titers were significantly different from those measured against the other five viruses (all had $P$ values of $<0.001$, Mann-Whitney rank sum test). Thus, although we have found clear evidence of antigenic differences among mumps virus strains, the fact that all sera neutralized all viruses supports the notion that mumps virus is serologically monotypic and argues against the evolution of exotic strains capable of escaping JL vaccine-induced immunity. However, the sera tested here were obtained from individuals 6 weeks after vaccination, a time when titers are relatively high (8), whereas numerous studies have found levels of MuV-specific antibody to decline significantly with time postvaccination (24, 25, 38, 54). This has been associated with decreased vaccine effectiveness (17, 31, 57) and increased odds of contracting disease (10, 19, 61). Thus, it is possible that by the time of adolescence (when antibody levels have declined) such antigenic differences may be of significance.

Of note, T cell immunity was not assessed in this study; thus, we cannot rule out the possibility that certain MuV strains might be capable of escaping vaccine-induced T cell responses. Given our evidence of effective B cell immunity shortly after vaccination, the ability to escape vaccine-induced T cell responses might not be of significance in the short term but could dramatically compound the problems caused by waning B cell immunity as the interval between vaccination and subsequent exposure increases. Also, it must be acknowledged that measurements of virus-neutralizing antibody *in vitro* may not be fully predictive of immunological activity *in vivo* given that numerous processes that occur in the host are not reflected in the assays used to measure virus viability *in vitro*.

It is important to highlight the fact that the occurrence of outbreaks in vaccinated populations is not a problem unique to the JL vaccine strain, given that outbreaks have also occurred in populations with a history of vaccination with the Urabe AM9 and Leningrad-Zagreb strains (3, 15, 18, 33, 46). Thus, development of new mumps vaccine strains, as some have suggested, is not a likely solution to the problem. Rather, revaccination during adolescence to combat waning immunity might be the most effective measure, as suggested by the experience with military recruits who were spared involvement in the mumps resurgence in the United States in 2006 despite belonging to the same age group and residing in high-density close-contact environments, conditions not dissimilar to those of university campuses where the bulk of the outbreaks occurred in 2006. The likely reason for this is that in 1991, the military had begun routine administration of MMR vaccine to recruits without regard to prior vaccination status. This policy was modified in 1995 and then again in 2006, but the end effect was that a significant proportion of recruits likely received a dose of mumps-containing vaccine upon entrance into the military (6).

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